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THE CENTROSOME IN THE MATURATION AND FERTILIZATION OF BULLA SOLITARIA.

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THE material upon which the following observations were made was collected at Woods Holl during the seasons of 1898–1900. The greater part of the work was done at the Marine Biological Laboratory under the direction of Dr. E. G. Conklin, and I take this opportunity of thanking him for his many valuable suggestions. I also wish to acknowledge my indebtedness to Dr. C. O. Whitman and Dr. C. W. Hargitt.

A fuller account of my observations both upon the subject of this paper and the cell lineage of Bulla, with a discussion of the pertinent literature, will be published later.

The sketches illustrating the mitotic changes in maturation were drawn from sections stained with Heidenhain's iron-alum followed by an aqueous solution of Bordeaux. In order to differentiate the sperm, it was necessary to use Conklin's mixture of haematoxylin and picric acid. The sperm, therefore, has been drawn from corresponding stages and inserted into these figures.

In the interpretation and nomenclature of the centrosome and sphere I have followed in the main Van Beneden. The term "centrosome" will be applied to the body which occurs at the pole of the spindle, etc., when that body has become differentiated into a central corpuscle, surrounded by a clear area, the medullary zone bounded by a definite wall. The body occurring at the center of the aster is the central corpuscle.

The central corpuscle becomes the centrosome. Sections of the ovotestis before copulation show the unfertilized egg lying free in the follicles of the hermaphroditic gland. The large germinal vesicle lies in the center of the egg; it contains a large vacuolated nucleolus, also basichromatin and oxychromatin granules. The deutoplasmic spheres are equally distributed in the cytoplasm and conceal its structure. I have not been able to discover any evidence of a central corpuscle or centrosome in the egg before mitosis begins. By the time the eggs are laid the first polar spindle is in the end of the prophase. In order to secure the earlier stages, a large number of animals were collected and killed as soon as they began to lay. The first polar spindle begins to form as the animals begin to lay. Sections of the ovotestis taken from animals killed while they were laying revealed the fact that every mature egg had already passed through the early prophase of the first spindle; even those eggs in the most distant follicles, where it is probable that the sperms from the receptaculum seminalis had not penetrated; I have found the sperms in the anterior part of the hermaphroditic duct, but not extending back to any considerable distance. The earliest stage thus far discovered had two well-formed central corpuscles and a definite central spindle connecting each, which passed through the germinal vesicle, the walls of which are plainly visible. The ring-shaped chromosomes have begun to form from the meshwork of linin and chromatin. The reduction of the chromosomes has not been worked out in detail. These ring-shaped chromosomes gradually take a deeper stain and come to lie in the equatorial plate of the first polar spindle (Fig. 1). In a cross-section of the equatorial plate I was able to count sixteen distinct chromosomes. There is a distinct cortical zone surrounding the central corpuscle. The astral rays pass through this clear area and extend to the central corpuscle. At this stage I have been able to

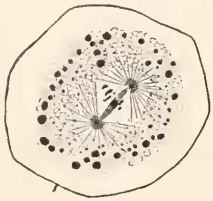

FIG. 1. — Taken from the ovotestis. Shows the central corpuscle surrounded by the cortical zone of the sphere. The central spindle is well formed and the chromosomes are forming into the first equatorial plate. Walls of the germinal vesicle still present, sperm entering at vegetal pole.

trace them nearly to the egg membrane. From now on, there does not appear to be any appreciable change in the mitotic figure, until after the egg has been laid, when it begins to migrate to the periphery of the egg, where it assumes a radial position. There is no difference in the character of the two poles of the spindle. During this movement of the spindle the chromosomes pass into the metaphase, and the centrosome becomes differentiated into a central corpuscle and a medullary zone which is limited by the walls of the old central corpuscle. This is the first time that we have a structure to which we can apply the term "centrosome" in the sense that I purpose to use the term. The cortical zone has enlarged and become much fainter. The chromosomes do not divide at once; the activity is centered in the centrosomes. While the chromosomes are still in the equatorial plate, the central corpuscle in each centrosome divides, having the dumbbell form. The centrosome rapidly increases in size, the periphery is limited by a definite line, which gradually becomes thinner. The medullary zone does not take a plasma stain, as it did in the previous stage (Fig. 9). The centrosome now begins to assume an elliptical form and at the same time to rotate. This rotation continues until the elongated centrosome, which encloses the second polar spindle, lies radially and in the same position that the first polar spindle did. The central corpuscles, connected by a central spindle, are so influenced by this elongation of the walls of the centrosome that they come to lie near the ends — at the foci of the ellipse. As the outer pole of the second polar spindle nears the periphery of the egg, the rays extend to the chromosomes, and they are pulled into the spindle to form the equatorial plate of the second polar spindle. It will be seen that in the main my results corroborate those of MacFarland,[1] Lillie,[2] and Conklin.[3]

The changes which take place in the centrosome during this stage are very interesting. The centrosome is so large and comes out with such perfect clearness that I have been able to

[1] "Celluläre Studien an Mollusken-Eiern," *Zoöl. Jahrb.* 1897.

[2] "Centrosome and Sphere in the Egg of Unio," *Zoöl. Bull.* Vol. i, No. 6. 1898.

[3] *Science*, March, 1898.

follow the details very carefully. The spindle is developed when the central corpuscles separate. At this time a very faint line can be seen connecting the new corpuscles; as the distance between them increases, the line becomes more distinct, until a central spindle can be clearly distinguished. In the mean time the line limiting the centrosome has become broken into pieces, which gradually become smaller and smaller until they cannot be distinguished from the granules of the cytoplasm. While these changes have been taking place, this broken line has served to mark the outer limit of the medullary zone. The old medullary zone has disappeared, and between the central corpuscle and the broken wall of the centrosome we have a new medullary zone, which is the cortical zone of the second polar spindle. The process is as follows: as the walls of the centrosome begin to break down, an area next to the central corpuscles and at each end of the centrosome appears; this begins to take a plasma stain (Fig. 3). The area gradually surrounds the central corpuscle and all of the space at the end of the spindle between the central corpuscle and the wall of the centrosome. In the mean time the central corpuscle has increased in size and is to become the centrosome of the second polar spindle; it ultimately becomes differentiated into a central corpuscle and a medullary zone.

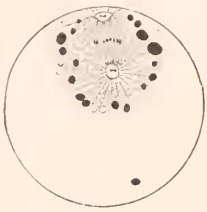

Fig. 2. — Metaphase of first maturation spindle. Two central corpuscles at each pole. Cortical zone limited by a dotted outline. The medullary zone does not take a plasma stain at this stage. Sperm head solid and elliptical.

Fig. 2 shows two central corpuscles in the centrosome at each pole of the spindle. Linville[1] shows a similar but not identical stage. The centrosome of the outer pole of the spindle does not enlarge more than is shown in the figure; as it reaches the surface of the egg, it breaks down and the central corpuscles form the division centers in the first polar body.

There is no telaphase in the first maturation spindle. The nearest approach to such a stage is shown in Fig. 3, where the

[1] "Maturation and Fertilization in Pulmonate Gasteropods," *Bull. Mus. Comp. Zoöl., Harvard.* Vol. xxxv, No. 8.

chromosomes have become partly hollow vesicles. A few of the interzonal fibers show at this stage, but they are very faint.

In the metamorphoses of the centrosome its attachment to the astral rays is plainly evident; the old rays can sometimes be seen in a stage younger than the one shown in Fig. 11, when the new rays have already begun to form and are attached to the central corpuscle. I believe that the rays of the first polar spindle disappear and that the rays of the second spindle rise *de novo*.

FIG. 3.— Prophase second maturation spindle. Wall of centrosome broken in pieces. Central corpuscles connected by a spindle. New cortical zone forming. Chromosomes are partly hollow. A few interzonal fibers are present.

The metakinesis of the second polar spindle takes place very rapidly. The chromosomes elongate, divide transversely, and as they move toward the poles, they assume a roundish form, and change into vesicular bodies which fuse to form the female pronucleus. During the time when they are fusing, the rays can be traced directly into the areas immediately surrounding them. In the stage of anaphase as represented in Fig. 5, the centrosome is evident, although it does not stain as deeply as in Fig. 4. Immediately after this stage the centrosome disappears and the cortical zone enlarges and completely surrounds the female pronucleus; later both male and female pronuclei come to lie in this clear area. A single centrosome passes off with the second polar body, which is much smaller than the one given off in the first polar body (Fig. 4).

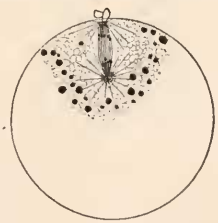

FIG. 4.— Anaphase second maturation spindle. Centrosome at each pole with a single central corpuscle. The medullary zone takes a plasma stain.

The eggs of this species are not especially favorable for a study of the problem of fertilization. During all of the earlier stages, the sperm head lies completely surrounded by deutoplasmic spheres. I have not been able to make out any continuous clear area about the sperm head during its progress through the egg. In one instance there was a definite clear area about the sperm nucleus after it had nearly approached the female pronucleus, otherwise it was unattended

by anything that corresponds to the "Hellerhof" of MacFarland and others. The sperm enters at the vegetal pole, though not in any definite place. The tail is lost before the sperm enters the egg membrane (Fig. 1). The head is a solid body having a distinct angle in the middle. If there is a middle-piece, it is practically indistinguishable. The only indication that I have found of such a body is that on one end of the sperm head sometimes one finds a narrow band that stains a little denser than the rest of the head.

The sperm head becomes top-shaped as it begins to migrate toward the animal pole with the point leading. The head becomes elliptical (Fig. 3) and stains very black. It remains in this solid form for some time, while the first polar spindle is passing from the metaphase until the anaphase of the second polar spindle. During the anaphase of the second maturation, the solid sperm head becomes vesicular, and for a very short time is accompanied by astral rays. I have not been able to discover a central corpuscle in connection with the aster, nor have I ever seen an amphiaster. At this same time secondary asters usually appear in the egg, which are smaller than the sperm aster. As the chromosomes of the second polar spindle begin to assume the vesicular form, the sperm aster disappears, and the sperm, consisting of one or more vesicles, rapidly approaches the inner pole of the second polar spindle. When the sperm consists of more than one vesicle, these fuse into one when the aster disappears. While the vesicular sperm is shifting its position it does not increase in size to any noticeable extent, but as soon as it comes near the female pronucleus, which now consists of but three or four vesicles, it rapidly increases in size until it is about twice as large as it was when migrating toward the animal pole. From the time that the sperm head enters the egg until it comes to lie in contact with the female pronucleus (Fig. 6), it is not attended, so far as I have observed, by any body which might be taken for a central corpuscle or a centrosome.

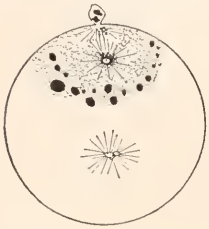

FIG. 5. — Late anaphase of second maturation spindle. Centrosome still present. Cortical zone enlarging. The sperm is composed of three vesicles and accompanied by astral rays.

The structure of the two pronuclei when they have come together (Fig. 6) is the same. The male pronucleus is usually regular in outline and slightly smaller. The irregularities in the outline of the female pronucleus often persist until the central corpuscles of the first cleavage appear. The chromatin stains very slightly and is connected by delicate linin threads. The changes through which the chromatin passes before the equatorial plate is formed may be described under three stages. First, the chromatin rapidly increases in staining power, forming a dense reticulum, often containing stellate masses of solid chromatin. Second, the chromatin begins to assume a definite form. The most conspicuous shape is the stage where the masses of chromatin have begun to break up into rings but are still attached to one another. The chromosomes have not yet become hollow. They stain uniformly. Third, the chromosomes have broken apart from each other, and have become hollow, round bodies. At first there is a delicate meshwork connecting them (Fig. 7), but this is soon lost and each pronucleus is filled with ring-shaped chromosomes. While the chromatin is undergoing the last two changes, the central corpuscles (the so-called cleavage centrosomes) of the first cleavage spindle make their first appearance. I have found them in a much earlier stage than the one figured, but in each case there was no connection between them; but these corpuscles with their rays have a definite relation with the pronuclei, that is to say, each pronucleus has an aster and central corpuscle with a faint cortical zone. My observations upon the cleavage centrosomes would tend toward the position, first, that they arise *de novo;* and, second, that one arises in connection with each pronucleus.

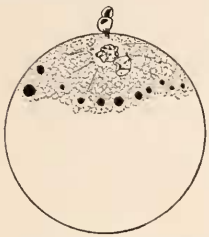

FIG. 6. — The female pronucleus is irregular in outline, surrounded by the cortical zone of the inner pole of the second maturation spindle. A few astral rays are present. Zwischenkörper of second polar body present.

FIG. 7. — Origin of cleavage centrosomes. One in connection with each pronucleus. The central corpuscles are surrounded by a faint cortical zone.

Metamorphosis of the Centrosome in Maturation.

The definiteness and clearness with which the several changes in the centrosome appear in Bulla make these changes the most important of the various stages in maturation and fertilization. In describing the changes of the centrosome, under various stages, I have no theoretical points in consideration. While the stages figured are clearly differentiated, still there are intermediate stages which graduate imperceptibly into one another.

In the earliest prophase that I found the central corpuscle was a large solid mass (Fig. 8). Surrounding the central corpuscle there was a conspicuous area, the cortical zone, which was sharply differentiated from the cytoplasm. The rays are not lost in the cortical zone, as MacFarland has shown for Diaulula, but extend to the central corpuscles, as Lillie has shown for Unio, and Linville for Limnaea. However, I do not find a row of microsomes, as in Unio, limiting the sphere, nor is the boundary formed by the fusing of the astral rays, as in Limnaea.

Second stage (Fig. 9). The central corpuscle has become clearly differentiated into a centrosome. It reacts to stain in a very different manner from what it did in a previous stage. There is now a medullary zone which takes on a plasma stain and is limited by a distinct line. The small dark body in the center is the new central corpuscle. The cortical zone has increased in size and is less easily distinguished from the surrounding cytoplasm. The line marking the periphery of the centrosome is the limiting wall of the enlarged central corpuscle of the previous stage.

Third stage (Fig. 10). The centrosome has increased in size. The line at the periphery is definite and whole. The central corpuscle of the previous stage has divided into two central corpuscles, which are connected from the first by faint lines. The medullary zone does not take a plasma stain. The cortical zone has become very faint and soon disappears as a distinguishable area in the cytoplasm.

Fourth stage (Figs. 3 and 11). The periphery of the centrosome loses its continuity, and openings occur in the wall; while

these changes in the wall are taking place, the centrosome becomes much enlarged and assumes an elliptical shape. Immediately after these breaks appear, there is a small part of the medullary area which takes a plasma stain. This area is somewhat triangular in shape and occurs at the end of the centrosome between the central corpuscle and the broken periphery of the centrosome. New astral rays are formed which extend to the central corpuscle. The old astral rays can be seen disappearing at this stage in the cytoplasm.

Fifth stage (Fig. 12). The central corpuscle of the second polar spindle has enlarged and is still undifferentiated. The pieces of the periphery of the old centrosome have become

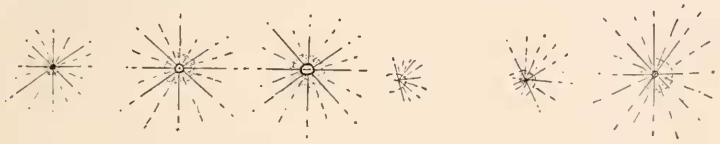

FIG. 8. FIG. 9. FIG. 10. FIG. 11. FIG. 12. FIG. 13.

FIGS. 8–13. — The changes through which the central corpuscle and centrosome pass from the prophase of the first maturation spindle to the metaphase of the second maturation spindle. In each case the solid dark body is the central corpuscle. The granular area surrounding the central corpuscle is the cortical zone. Figs. 9, 10, and 13 show a complete centrosome, having a central corpuscle and medullary zone.

smaller. There is now a distinct cortical zone around the central corpuscle, which has been derived from the medullary zone of the centrosome of the first polar spindle. This stage is identical with the first one described, except that the rim of the old centrosome is still present and the central corpuscle is only about one-half as large.

The sixth stage (Fig. 13) shows the rim of the old centrosome still present, but in smaller pieces which do not stain as deeply as in the previous stage. The cortical zone has enlarged and become fainter. The centrosome is composed of a medullary zone and a central corpuscle.

Summary.

The central corpuscle of the first polar spindle becomes the centrosome of the second polar spindle with a medullary zone and a central corpuscle. The medullary zone of the centro-

some of the first polar spindle gives rise to the cortical zone of the second polar spindle. The central corpuscle of the centrosome of the first polar spindle gives rise to the centrosome of the second polar spindle. Thus we may say that the centrosome of the first polar spindle in Bulla solitaria not only gives rise to the centrosome and mitotic figure of the second polar spindle, but to the cortical zone or outer sphere substance surrounding each centrosome.

Allegheny College,
October, 1900.